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Dear colleagues from Romania and abroad

It is our pleasure to invite you to attend at the 30th edition of the International Conference Progress in Organic and Macromolecular Compounds, MACRO Iasi 2025, a traditional event organized by the Petru Poni Institute of Macromolecular Chemistry, between 23 and 26 September 2025, in Iasi.

The International Conference addresses polymer and organic chemists and physicists from academia, research institutes and industry, being intended as a dynamic platform for the presentation and sharing of their research and ideas.

MACRO Iasi 2025 gives a broad overview of major topics in organic and polymer synthesis and physics, multifunctional polymeric architectures, engineering of polymeric materials and their applications.

Also, as part of the MACRO Iasi conference, the workshop "POLYSACCHARIDE BASED (BIO)HYBRID NANOSTRUCTURES" (September 23, 2025) will be organized, to which you are welcome to participate (please contact the organizers - hybsac.pnrr@icmpp.ro).

This meeting could not have been organized without the generous and tireless support and contribution of many individuals and groups within and outside the ICMPP. Therefore, we would like to acknowledge to all the invited lecturers, speakers, board and committee members, chairpersons, sponsors and all the people that have been involved in the organization and presentation of relevant results and perspectives.

Best wishes for a professionally rewarding conference!

Valeria HARABAGIU and Marcela MIHAI

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PROJECTS

POLYMER-PROTEIN COMPLEXES AS VERSATILE CARRIERS FOR TARGETED PROTEIN AND DRUG DELIVERY CHARACTERIZED BY SMALL-ANGLE NEUTRON SCATTERING

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1. Introduction

Micro- and nano-gels made from biopolymers such as polysaccharides combine the properties of nanoparticles and hydrogels and respond to external stimuli such as pH, ionic strength or temperature (T) as their network structure enables swelling/deswelling transitions and they possess the appropriate dimensions for encapsulation and nano-delivery. The addition of proteins to polysaccharides and their coupling to form more complex morphologies opens up possibilities for fine-tuning gel properties by physically cross-linking the components and modifying the gel network structure. In general, complexes of proteins with natural or biodegradable synthetic polymers can result in micelles, vesicles or more complex morphologies. These systems find application in nanomedicine for the targeted delivery and controlled release of proteins thanks to the pH-responsiveness of the formed structures. Moreover, the presence of a polymer/protein layer enables the encapsulation of ionic drugs within such particles. Furthermore, proteins protect bio-active molecules such as vitamins through non-covalent interactions. Vitamins D2 and D3 (VD2 and VD3) play an important role in human and animal nutrition by regulating the circuit of calcium and phosphorus cycling in the body and incorporation into the skeleton. Challenges of administrating vitamins D include its poor water solubility, chemical degradation at elevated temperatures, and variable oral bioavailability. Micro- and nanogels composed of polysaccharides and various proteins protect vitamins D from degradation than the protein alone, which is susceptible to structural changes triggered by modifications in its environment, such as pH and T.

Understanding the physicochemical properties of these polymer-protein complexes under different conditions, with and without encapsulated components, is therefore crucial for their optimization and utilization. Small-angle neutron scattering (SANS) provides comprehensive information about the meso- and nanoscale structures of such materials. The particularly strong difference in the neutron scattering cross section between the hydrogen isotopes protium (^{1}H) and deuterium (^{2}H or D) offers the unique advantage of D-labeling (contrast variation) of hydrocarbon materials such as polymers and proteins. This work demonstrates how contrast variation SANS can uniquely resolve the complex structure and morphology under application-relevant pH and T conditions in polymer-protein complexes based on bio- and synthetic polymers with encapsulated drugs or vitamins.

2. Experimental

This report covers experimental SANS studies on complexes of biodegradable polymers such as PEO-PDMAEMA or PEO-QPDMAEMA and insulin [1], as well as on biopolymers such as different types of carrageenan and BSA or HSA proteins. Complexes encapsulating drugs such as protoporphyrin-IX or VD3 vitamins have also been investigated. SANS experiments are presented that were performed under contrast variation conditions over a wide Q range between $2\text{x}10^{-4}$ and 1.0 Å^{-1} at small-angle diffractometers installed either at stationary (reactor) or pulsed (spallation) neutron sources. This approach covered a broad length scale in real space between a few Å and mm. To apply the contrast variation method selected components in the complex morphology in a partially or fully deuterated state were used. Neutron contrast conditions were used with an appropriate mixing of deuterated and protonated components in aqueous solutions,

allowing either full contrast, thus the full morphology "visible" in D_2O when all components were used in protonated state; the visibility of the PE block in 70% D_2O - 30% H_2O when deuterated QPDMAEMA block was matched out; or protonated protein, which was matched out in 35-40% D_2O content, thus only the polymer was visible. Samples were analyzed under different pH, temperature and concentration conditions. Scattering patterns were interpreted using appropriate structural models to extract the shape, size and density of the polymer-protein morphologies. Encapsulation and controlled drug release was checked by fluorescence spectroscopy. To support the structural findings, cryo-TEM was also used for some of the complexes in addition to neutrons.

3. Results and discussion

Figure 1a shows the scattering patterns of a sample containing the diblock copolymer with a weakly charged block and insulin in D_2O for two pH conditions. Scattering of polyelectrolyte-protein complexes is observed at pH = 7.4, whereas at pH = 11, superposition of scattering of the two components individually is observed, since no complex is formed at this pH.

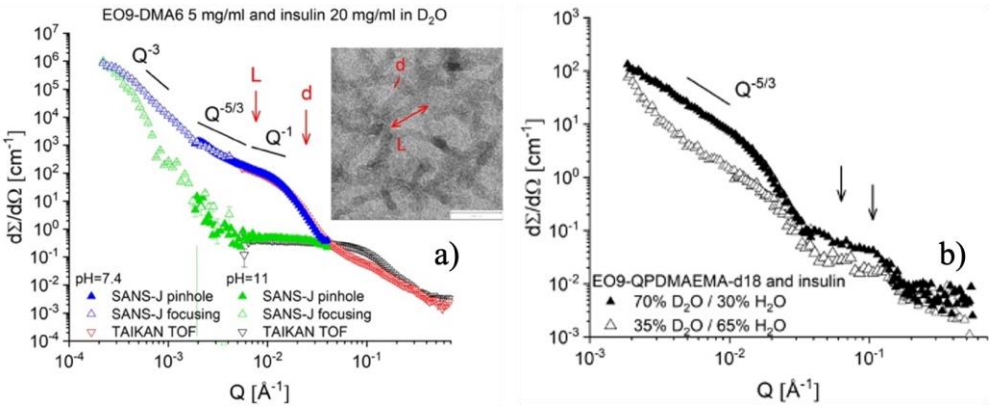

Figure 1. Scattering patterns from the combination of weakly charged diblock copolymer and protein in full contrast (a) and selected contrast matching conditions (b). The cryo-TEM image is shown as an inset in (a). The power law behavior of the scattering and the estimated thickness and length of the complex morphology are indicated in (a). Arrows in (b) indicate the scattering features representing correlation effects between the charged components of the complex.

Data collected with two different instruments at reactor (SANS-J) and spallation (TAIKAN) sources in Japan sources are shown in parallel using different color scheme. Very good agreement is observed between the scattering data at medium and low Q and the cryo-TEM images (inset in Figure 1a) regarding the medium- and large-scale morphology of the polyelectrolyte-protein complexes: one-dimensional flexible morphologies are formed (Q^{-1} power law behavior of the scattered intensity), which, on a larger length scale, behave like a branched morphology resembling that of a solvent-swollen self-avoiding polymer coil ($Q^{-5/3}$ power law). The thickness and segment length can be estimated from the analysis of the cryo-TEM image, while the power law behavior of the scattering patterns at medium Q and pH = 7.4 agrees well with the morphology revealed by the micrograph. At low Q, an increase in the scattering pattern is observed under both pH conditions, presumably due to the association of the one-dimensional flexible morphologies into a larger network. The contrast matching results (Figure 1b) for the QPDMAEMA block (full symbols) or insulin (open symbols) confirm the morphology formation scenario described above: over an extended Q range, a $Q^{-5/3}$ power law behavior is observed when alternately visualizing either the charged block or the protein, i.e. both oppositely charged species jointly assemble the complex, while the PEO block forms a kind of shell and network of the morphology. The broad peak-like scattering feature at high Q appears under both contrast conditions, albeit with different intensities, indicating correlation effects between the interacting charged components in the complex system, which are made visible or invisible in the scattering

experiment depending on the contrast condition.

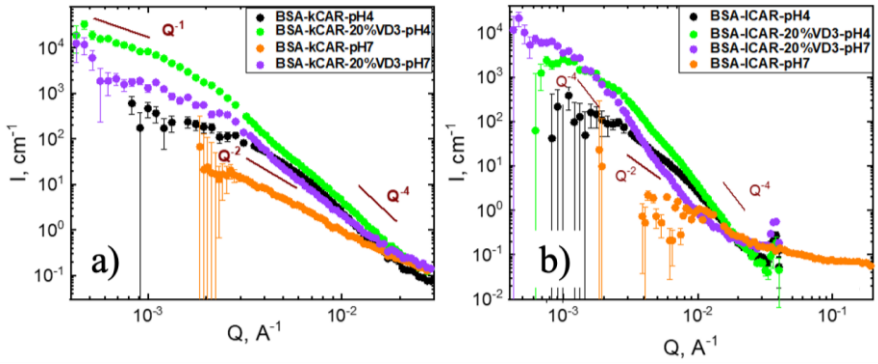

Figure 2. SANS profiles from (a) BSA-k-carrageenan complexes and (b) BSA-λ-carrageenan complexes upon 20% VD3 encapsulation and pH adjustment from pH = 4 to pH = 7.

Figure 2 shows the scattering patterns of two systems containing BSA protein and either the polysaccharide k-carrageenan (a) or λ-carrageenan (b) at two pH conditions, pH = 4 and pH = 7, and with 20% VD3 encapsulation. It shows that the addition of VD3 led to the stabilization of larger complex morphologies, likely because VD3 acts as an additional cross-linking point for association of already formed complexes or induces further structural rearrangements within the BSA component. Furthermore, SANS data showed complex disruption at pH = 7, as indicated by the significant drop in scattering intensity (orange symbols). However, when VD3 was encapsulated in the complexes, increasing the pH did not lead to complete disruption, as scattering from large aggregates is still visible at low Q, suggesting that VD3 increases the stability of the complexes. This stabilization effect was more pronounced in case of λ-CAR complexes than in case of κ-CAR complexes, since even at pH = 7, larger stable morphologies could be identified based on the strong scattering observed at low Q.

4. Conclusions

The application of contrast variation SANS to investigate the structure and morphology of polymer-protein complexes and their drug encapsulation properties using biopolymers such as carrageenan polysaccharides or synthetic PEO-based di-block copolymers is described in details.

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MULTIFUNCTIONAL POLYSACCHARIDE-BASED HYBRID HYDROGELS WITH POROSITY TAILORED BY CRYOTROPIC GELATION

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1. Introduction

Hydrogels are three-dimensional polymer networks that have attracted significant attention due to their structural similarity to living tissues and their responsiveness to environmental stimuli [1]. These features make them valuable for a wide range of biomedical, environmental, and technological applications [1]. However, conventional hydrogels are often limited by poor mechanical strength and slow responsiveness, which restrict their broader use. To overcome these challenges, hybrid hydrogel architectures and cryotropic gelation strategies have been developed to introduce multifunctionality, mechanical stability, and tailored porosity [2,3]. Cryogenically structured hydrogels, in particular, exhibit unique features such as high elasticity, toughness, rapid water sorption, and interconnected macroporosity that support efficient mass transport without diffusion-related issues [2].

In this lecture will be discussed the development of multifunctional polysaccharide-based hybrid cryogels, with focus on three main categories: (i) interpenetrating polymer networks (IPNs), in which polysaccharides are integrated into synthetic networks to generate mechanically resilient and porous materials; [2,4,5]; (ii) polysaccharide–inorganic filler hybrids, engineered for selective sorption, pollutant removal, and catalytic properties [6-8], and (iii) polysaccharide–plant extract hybrids, where bioactive phytocompounds impart antioxidant, and antimicrobial properties with applications ranging from healthcare to food packaging [9-14].

By combining semi-IPN strategies, cryotropic gelation, and functional biopolymer–filler integration, we demonstrate how porosity can be engineered to yield advanced hybrid hydrogels tailored for applications in medicine, environment, and food systems.

2. Results and discussion

Interpenetrating polymer networks (IPNs)

Semi-IPN cryogels formed by embedding polysaccharides such as dextran, dextran sulfate, and chitosan (CS) into cross-linked polyacrylamide matrices exhibit finely controlled porosity and ultra-fast swelling, making them promising for drug delivery and environmental remediation [2]. More complex hybrid architectures were obtained by introducing triple-cationic systems (CS, polyethyleneimine, and PDMAEMA) reinforced with hydrated iron oxide nanoparticles, achieving remarkable phosphate sorption capacity, and recyclability [4]. Similarly, tricomponent polyelectrolyte complex cryogels combining CS, ionene polycations, and carboxymethylcellulose efficiently removed oxyanions and heavy metal ions while exhibiting complete antibacterial activity against both Gram-positive and Gram-negative bacteria [5].

Polysaccharide–inorganic filler hybrids

Polysaccharide–inorganic filler hybrids, particularly CS-based cryogels, have been also developed as multifunctional platforms for both controlled drug release and environmental remediation. Incorporation of natural zeolite produced hybrid networks with adjustable morphology, swelling behavior, and drug release kinetics, while ion-imprinted cryogels offered highly selective and reusable sorbents for removal of Cu^{2+} ions [6]. Functionalization with aminopolycarboxylic acids further extended the removal spectrum to Pb^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} ions, maintaining efficiency in both batch and continuous column operations, including

real wastewater treatment (Figure 1A,B) [7,8].

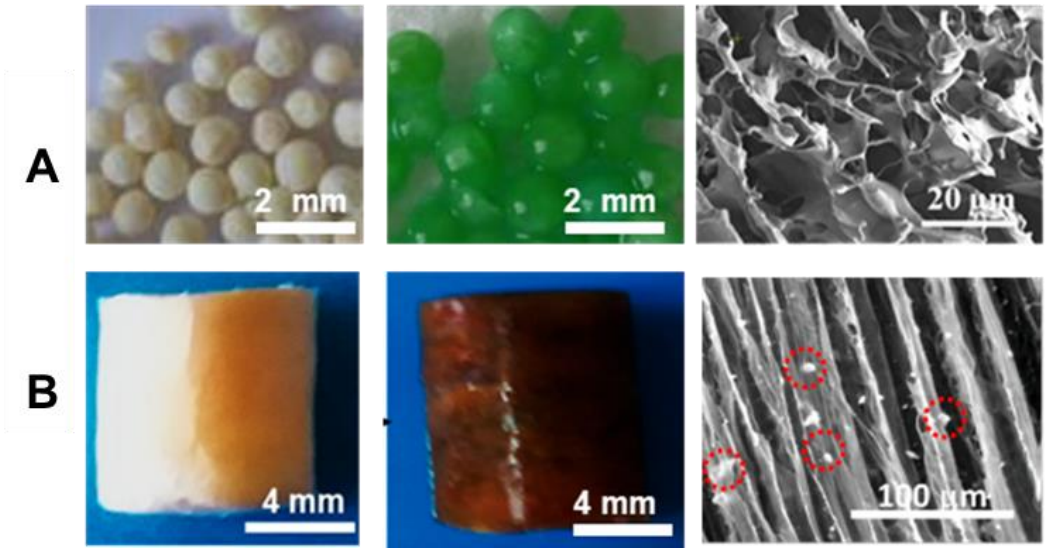

Figure 1. (A) Aminopolycarboxylic acid–functionalized CS-based composite cryogels with heterogeneous morphology, used as beads for selective sorption of Cu^{2+} ions from aqueous solutions; (B) CS–acid-activated zeolite hybrid cryogels with anisotropic pores, employed as monoliths for the simultaneous removal of metal ions from industrial wastewater.

Maximizing resource efficiency and transforming waste into high-value products for new applications represent central principles of circular economy. Thus, thiourea-functionalized CS cryogels, after Cu(II) or Ag(I) uptake were converted into Cu- and Ag-nanoparticle-loaded cryogels, yielding recyclable and highly active catalysts for 4-nitrophenol hydrogenation.

Polysaccharide–plant extract hybrids

Hybrid hydrogels that integrate mechanical stability with antioxidant, antimicrobial, and sensor functionality were also developed by the incorporation of bioactive plant extracts into polysaccharide cryogels. CS/dextrin cryogels loaded with *Thymus vulgaris* essential oil showed a more than fortyfold increase in elasticity compared to neat polysaccharide films, while providing significant antifungal and antioxidant activity [9]. Xanthan/PVA cryogels containing red grape pomace extracts demonstrated polyphenol-mediated pore stabilization, enhanced mechanical performance, and strong bioactivity, supporting applications in food packaging [10]. For healthcare applications, thiourea–CS cryogels enriched with *Hypericum perforatum* extracts exhibited outstanding liquid uptake, mechanical resilience, antioxidant potential, and broad-spectrum antibacterial activity [13]. Most recently, anthocyanin-rich bilberry extracts were incorporated into xanthan-based cryogels, producing ultra-light, highly porous constructs with antimicrobial activity, antioxidant capacity, and pH-responsive color changes [14].

3. Conclusions

Our research demonstrates that the combination of polysaccharides with synthetic polymers, inorganic fillers, or plant-derived bioactive compounds through cryotropic gelation enables the creation of multifunctional hybrid hydrogels with precisely engineered porosity. IPN-based architectures provide resilience and sorption of multiple contaminants; polysaccharide–inorganic hybrids enable both pollutant remediation and catalytic valorization, while polysaccharide–plant extract hybrids bridge biomedical, food, and environmental domains with bioactivity and visual monitoring functions.

Acknowledgements

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REACHING FOR THE STARS WITH NEW GENERATIONS OF FUNCTIONAL POLYMETHACRYLATES

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1. Introduction

In the recent years, significant progress has been achieved in the precise synthesis of star polymers with tailored functional arms and the development of effective strategies for their covalent immobilization on solid substrates enabling their successful application in biomedical field. Concurrently, the development of efficient systems for protecting genetic material from degradation and for delivering nucleic acids to specific targets still remains a major challenge in gene transfection research. While viral vectors have demonstrated promising outcomes, their use is often limited by immunogenic side effects, highlighting the need for safe and less pathogenic synthetic alternatives.

Polycationic polymers have emerged as a promising solution, as their amino groups enable the condensation of DNA or RNA into compact biohybrid structures known as polyplexes. Recent advances in the synthesis and detailed characterization of star-shaped polymers, along with their immobilization onto functional polymer surfaces, have facilitated the creation of star polymer-based vectors for gene therapy. Among these, polycationic star polymers constructed from poly(N,N-dimethylaminoethyl methacrylate) (PDMAEMA) have been the most extensively investigated.

2. Results and discussion

Star-shaped amino-functionalized polymethacrylates were synthesized using modern controlled polymerization techniques, and their solution behavior under varying pH and temperature conditions was systematically investigated to assess their potential biomedical applications. A series of star polymers with a hyperbranched poly(arylene oxindole) core and PDMAEMA arms differing in molar mass and arm length was obtained using atom transfer radical polymerization (ATRP).

Dynamic light scattering measurements revealed that stars existed as single macromolecules in acetone, while small aggregates were observed in aqueous or alcoholic media, with sizes remaining suitable for biomedical use. PDMAEMA stars were found to be pH-sensitive, with higher pH reducing positive charge and decreasing nanostructure size. At pH 13, uncharged stars exhibited thermosensitivity, and phase transition temperatures decreased with increasing arm length.

Obtained polycationic star polymers electrostatically bound negatively charged nucleic acids to form compact polyplexes, enabling safe and efficient genetic material delivery into cells. Polyplexes reached a constant size of approximately 150 nm above N/P = 6. Cryo-TEM imaging showed that stars with shorter arms formed elongated clusters, whereas longer-arm stars produced larger, regular spherical polyplexes. Transfection efficiency, assessed via luciferase activity, correlated with polymer cytotoxicity, and longest-arm stars provided maximal gene expression while maintaining full cell viability [1]. To reduce cytotoxicity, non-ionic di(ethylene glycol) methacrylate (DEGMA) was incorporated into the arms, yielding smaller and less compact polyplexes with significantly reduced cytotoxicity and high gene expression at elevated N/P ratios [2].

Over the past decade, research on nano- and microstructured star polymer layers on solid surfaces has advanced considerably. This progress includes the precise synthesis of star polymers with tailored

functional arms, the development of effective immobilization strategies, comprehensive characterization of the resulting layers, and their successful bioapplication [3].

Therefore, in our further research we obtained the layers composed of poly(oligo(ethylene glycol) methacrylate) (POEGMA) stars which supported fibroblast and HT-1080 cell growth, with noninvasive detachment controlled solely by temperature changes. Detachment from star POEGMA layers occurred faster than from linear brushes [4]. These thermoresponsive layers were subsequently used for deposition of DNA-polymer carriers, enabling polyplex formation and efficient nucleic acid delivery, with transfection efficiency several times higher than controls. Detached transfected cells maintained viability and adherence upon reseeding [4].

Finally, for further development of research on functional polymethacrylates, POEGMA-O star polymers with functionalities of 4 and 12 were grafted onto modified silicon surfaces and silicon-based sensors used for QCM studies. Nanolayers were characterized by ellipsometry, AFM, and contact angle measurements, confirming high homogeneity and increased hydrophilicity with increasing arm number and polymer molar mass. Protein adsorption tests using lysozyme and fibrinogen demonstrated significantly enhanced resistance to nonspecific interactions, particularly for 12-arm polymers, which achieved up to 100% protein removal. These findings highlight the potential of POEGMA-OH star polymers as effective antifouling layers for biomedical and sensor applications.

3. Conclusions

Functional methacrylate-based star polymers provide versatile platforms for biomedical and sensor applications. PDMAEMA stars enabled efficient, safe gene delivery, with DEGMA incorporation reducing cytotoxicity and enhancing transfection. POEGMA star nanolayers exhibited homogeneity, thermoresponsiveness, and strong antifouling properties, improved by higher arm number and molar mass. These findings demonstrate that functional star polymethacrylates, with tunable architecture and chemistry, offer multifunctional nanomaterials for gene therapy, tissue engineering, and antifouling surfaces.

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FROM TREES TO TECH: THE SILENT REVOLUTION OF CELLULOSE NANOFIBERS IN BIOELECTRONICS

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1. Introduction

The rapid growth of the bioelectronics sector has ushered in a new era of functional devices that seamlessly interface with biological systems. From wearable sensors to implantable neural probes, the demand for flexible, biocompatible, sustainable, and electrically conductive materials has never been greater [1]. Traditional electronic materials, which are often based on rigid synthetic polymers or scarce metals, struggle to meet these requirements, especially with regard to long-term environmental and biomedical compatibility. This has driven the exploration of bio-derived alternatives, with cellulose nanofibers (CNFs) emerging as a promising option [2].

Cellulose is the most abundant biopolymer on Earth [3]. It is a structural component of plant cell walls, and it is naturally renewable, biodegradable, and non-toxic. Through controlled mechanical, enzymatic, or chemical processing, cellulose can be deconstructed into nanoscale fibers with diameters typically below 100 nanometers (nm). These cellulose nanofibers (CNFs) exhibit a unique combination of high mechanical strength, a large surface area, tunable surface chemistry, and optical transparency. These properties position CNFs as versatile building blocks for next-generation bioelectronic devices. CNFs can be processed into flexible films, aerogels, hydrogels, and composite structures, enabling integration into diverse device architectures [4]. In addition to their attractive mechanical and environmental attributes, CNFs offer functionalities that are highly relevant to bioelectronics. Their hydrophilic and porous nature allows them to interact closely with cells, tissues, and ionic environments - a prerequisite for stable bioelectronic interfaces. Moreover, CNFs can be chemically modified. They can also be combined with conductive polymers, nanoparticles, or carbon-based nanomaterials. This combination can impart electronic conductivity. They can do so without compromising their intrinsic sustainability and biocompatibility. Recent advances for wearable sensors, biodegradable electrodes, ion-conducting membranes, and implantable devices have underscored the broad technological potential of CNF-based substrates. The silent revolution of cellulose nanofibers (CNFs) lies in their ability to transform an age-old natural resource into cutting-edge technological solutions. By bridging the gap between biological systems and electronics, CNFs represent a paradigm shift in material design, merging sustainability with performance.

This paper explores the progress, challenges, and future opportunities of CNFs in bioelectronics, emphasizing how this tree-derived material is poised to transform the landscape of sustainable electronic technologies.

2. Experimental

The cellulose sources employed for the preparation of cellulose nanofibers (CNFs) included microcrystalline cellulose (MCC; Avicel PH-101, Sigma-Aldrich, 99% hydrolyzed, crystallinity index (Cr.I.) = 78, degree of polymerization (DP) = 140), cotton linter (CL; microcrystalline cellulose powder, 20 μm, Sigma-Aldrich, Cr.I. = 86, DP = 465, viscosity = 8.4 cPs), eucalyptus pulp (EYPT; commercial bleached eucalyptus cellulose, Sodra Company, Sweden, Cr.I. = 52, DP = 2742, viscosity = 865 dm^3/kg), and α-cellulose (α-CEL; powder, Sigma-Aldrich, purity 95%, Cr.I. = 68, DP = 950). The reagents used for TEMPO-mediated oxidation included 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO; Sigma-Aldrich), sodium bromide (NaBr; Sigma-Aldrich), and an 8% sodium hypochlorite solution (NaClO; Chemical

Company). All chemicals and solvents were utilized as received, without further purification. To fabricate the highly stretchable, self-healing, and conductive hydrogel, 4-(bromomethyl)phenylboronic acid (PBA) and 1-vinylimidazole were sourced from Sigma-Aldrich and applied directly without additional purification. Ethyl acetate, acrylamide (AM), N,N′-methylene bisacrylamide (MBA, 97%), ammonium persulfate (APS, 99.5%), and N,N,N′,N′-tetramethylethylenediamine (TEMED, ≥99.5%) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd.

3. Results and discussion

The scientific literature contains a wide variety of experimental approaches that report the production of nanoscale cellulose derivatives, including nanocrystalline and nanofibrillated cellulose, using sources that are exotic in some areas of the world but common in others. Among these categories of natural materials, non-agricultural sources play a special role (Figure 1). These sources include tunicates and red algae, as well as agricultural sources such as spinifex grass, miscanthus, coconut coir, and pineapple leaves. To eliminate problems related to immense source variability and ensure better data reproducibility, we opted to use commercial cellulose sources available to anyone, which provide predictable results.

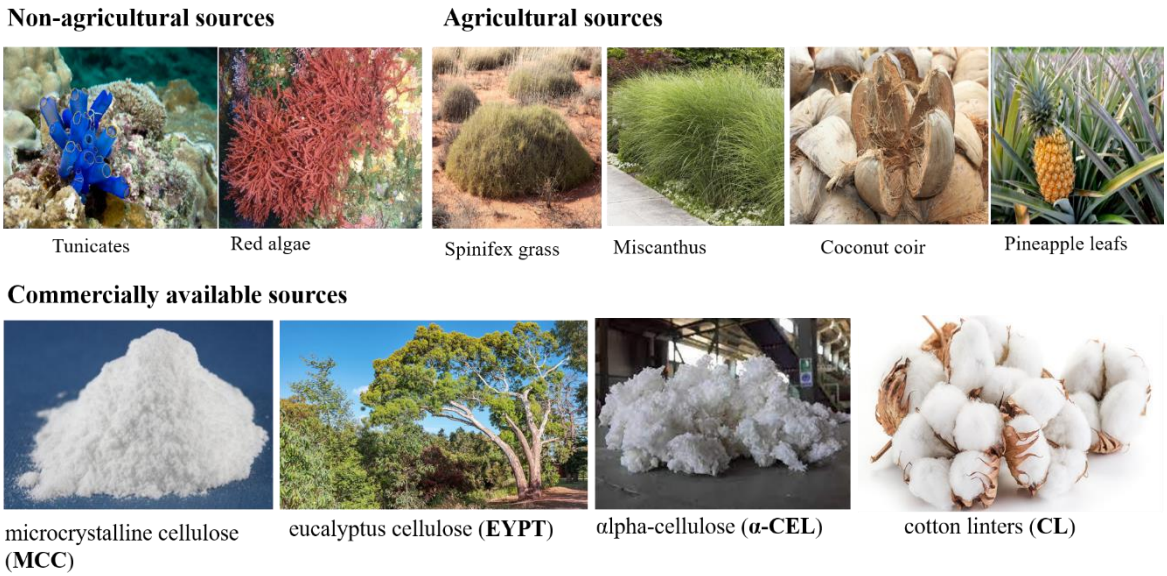

Figure 1. The different cellulose sources used in the preparation of fibrillated nanocellulose, including non-agricultural sources, agriculturally derived sources, *versus* commercially available sources.

Through the use of pretreatment processes followed by chemical oxidation using the TEMPO-NaBr-$NaClO_4$ system, the nanofibrillated cellulose fractions could be isolated from each of the starting materials, each with their own particular morphological and structural characteristics. By incorporating cellulose nanofibers (CNFs) into a system containing acrylic monomers and tailor-made ionic liquids, a robust cross-linked hydrogel network can be constructed. The structural integrity and multifunctionality of the hydrogel arise from the integration of multiple crosslinking mechanisms, including dynamic covalent boronic ester bonds, noncovalent interactions (hydrogen bonding and electrostatic forces), and chain entanglements within the interpenetrating CNF-based polymer networks. This synergistic combination provides an optimal balance between mechanical performance and multifunctional properties.

Furthermore, the coexistence of conductive ionic liquids and negatively charged surface carboxylate groups on CNFs facilitates ion transport by establishing efficient conductive pathways. Owing to its homogeneous microstructure, the hydrogel exhibits high optical transmittance (>95%), which is critical for optical signal accessibility. Leveraging these features, the tailor-made ionic liquid serves not only as a structural

component but also as a functional element for constructing high-performance ionic conductive hydrogels (ICHs). The resulting ICH-based sensors demonstrate reliable, sensitive, and stable monitoring of human motion. Overall, this study highlights a versatile design strategy for developing next-generation intelligent sensors and electronic skins.

4. Conclusions

One objective of this study was to optimize the isolation of carbon nanofibers (CNFs) from four commercially available cellulose sources by integrating TEMPO-mediated selective oxidation with alkaline–acid and ultrasonic pretreatments, and to elucidate how the structural characteristics of the resulting CNFs influence their self-assembly behavior. Our results demonstrate that the dimensions, morphologies, and surface carboxyl group densities of the CNFs are strongly dependent on the cellulose precursor.

A second objective was to incorporate CNFs into hydrogel-like architectures using ionic liquids functionalized with boron-containing moieties, thereby imparting enhanced functionality and structural versatility. The resulting hydrogels exhibited outstanding electrical and mechanical performance, including ultra-high extensibility, elevated tensile strength, pronounced viscoelasticity, intrinsic self-healing capability, and reversible adhesion. Furthermore, experimental investigations revealed that the synergistic combination of these features enables the fabrication of ionically conductive hydrogels that function as highly sensitive, stable, and reproducible wearable sensors capable of detecting and discriminating diverse human motions in real time.

Acknowledgements

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CHITOSAN-*g*-POLY(*N*-ISOPROPYLACRYLAMIDE) POLYPLEXES WITH DNA MOLECULES OF DIFFERENT LENGTHS

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1. Introduction

Non-viral drug and gene delivery systems utilizing polymers, liposomes, and nanoparticles have gained scientific interest due to their lower immunogenicity, increased safety, low cost, and ease of manufacture [1]. Natural polysaccharides like chitosan are promising non-viral vectors due to their biocompatibility, biodegradability, low toxicity, and cationic charge. Chitosan's positive charge allows binding of negatively charged genetic material, while also facilitating cellular uptake and transport. Its chemical modification with amino and hydroxyl functional groups enhances carrier performance [2]. Grafting with natural or synthetic polymers improves adhesive properties, water solubility, and therapeutic applications. Temperature-responsive poly(*N*-isopropylacrylamide) (PNIPAM) is a notable example of grafted polymers that offer additional functionalities and responsiveness to external stimuli [3].

2. Experimental

This study focuses on the co-assembly of a chitosan-*graft*-poly(*N*-isopropylacrylamide) (Chit-*g*-PNIPAM) copolymer with DNA molecules of different lengths (i.e., 50 or 2000 bp) to create polyplexes that could be used as gene delivery systems [4]. The chitosan's amino groups facilitate electrostatic interactions with the negatively charged phosphate groups of DNA molecules, while the PNIPAM side chains provide thermoresponsive properties to the assembly. Various N/P (amino to phosphate groups) mixing ratios were tested to form stable polyplexes. The mass, size, size distribution, and effective charge of the resulting nanoassemblies were analyzed using dynamic and electrophoretic light scattering (DLS and ELS), and their morphology was examined through electron microscopy (STEM). The polyplexes' response to environmental changes, such as temperature and ionic strength, and their stability in biological media were also assessed. The DNA binding affinity of the graft copolymer was evaluated using fluorescence spectroscopy and EtBr quenching assays, while the structure of the complexed DNA chains was investigated by infrared spectroscopy.

For the preparation of the polyplex solutions, appropriate volumes of a 1.4 mg/mL DNA50 or 2000 solution were added to 1 mL of a 1 mg/mL Chit-*g*-PNIPAM solution, and the final volume was adjusted to 5 mL. This way, the concentration of the graft copolymer is kept constant throughout the series of samples, while the N/P ratio ranges from 0.5 to 4. In some cases, partial precipitation was observed, and the corresponding measurements were performed on the supernatant.

3. Results and discussion

As seen in Figure 1, for both DNAs, an increase in mass (proportional to scattered intensity) and charge neutralization was observed as the concentration of DNA increases (or equivalently, the N/P ratio decreases). This denotes an increased interaction or aggregation, eventually leading to precipitation at low N/P values. For N/P above one, soluble or stable polyplexes with lower mass and increased positive charge are formed, apparently due to the contribution of the Chit-*g*-PNIPAM copolymer. Regarding the effect of DNA length, the polyplexes formed with the long DNA have greater mass or density and less charge,

indicating a stronger interaction with the graft copolymer. Regarding the corresponding sizes (DLS size distributions), the Chit-*g*-PNIPAM copolymer exhibits two peaks, with the larger one ranging from 300 to 600 nm, which suggests some degree of self-aggregation in solution. For the short DNA sample, at high N/P values, two peaks are observed, attributed to polyplexes formed with the corresponding species of the grafted chitosan, while at N/P equal to 1, only one peak is discerned, indicating denser or more compact structures. For the long DNA, only one peak at about 75 nm is observed in all cases, which implies the formation of more compact structures, also denoting increased interaction between the two components.

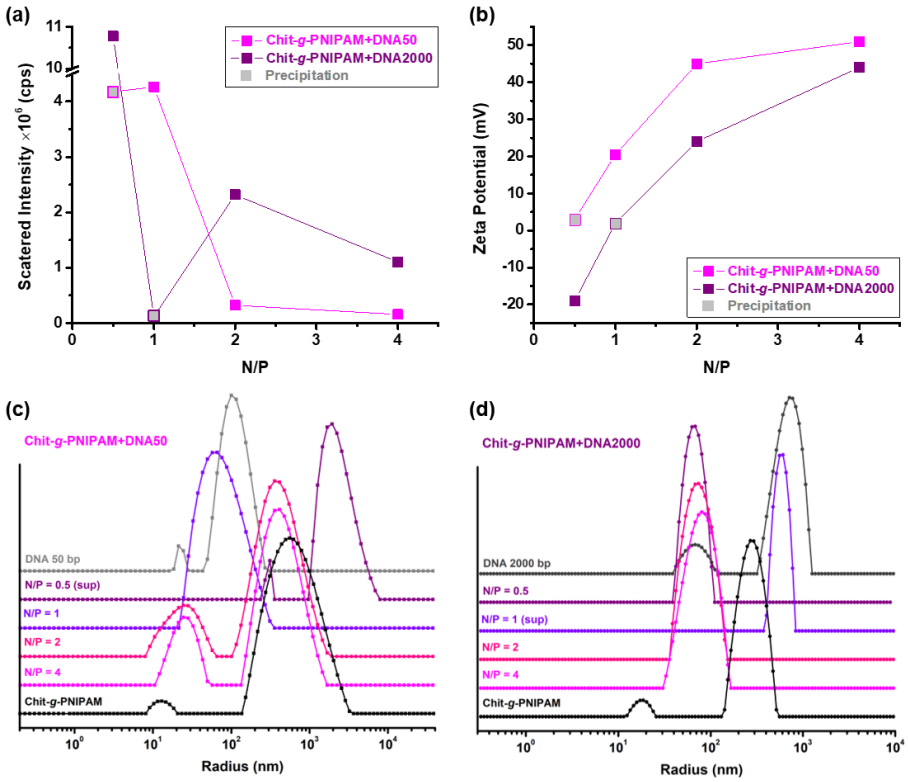

Figure 1. DLS and ELS results regarding: (a) scattered intensity; (b) zeta potential; (c, d) size distributions, for the polyplexes of the two Chit-*g*-PNIPAM+DNA systems. Adapted from [4].

The morphology of the polyplexes was visualized by STEM, with the obtained images for both DNAs shown in Figure 2 indicating the existence of spherical, homogeneous nanostructures of various sizes and relatively loose conformation. The size of the nanostructures for the long DNA sample is smaller than that for the short one, verifying once more the formation of more compact structures.

The polyplexes exhibited thermoresponsiveness above 35 °C as thermally triggered aggregation takes place, due to the presence of PNIPAM. This is evidenced by a significant increase in intensity, which is more intense in the case of the short DNA sample. From the corresponding changes in the size distributions (Figure 3), the formation of more compact structures (due to hydrophobic interactions) is observed for the short DNA sample. For the long DNA, no significant change in size occurred, most probably because of the already increased initial compactness of these polyplexes. Nevertheless, the transitions are fully reversible upon cooling back to 25 °C.

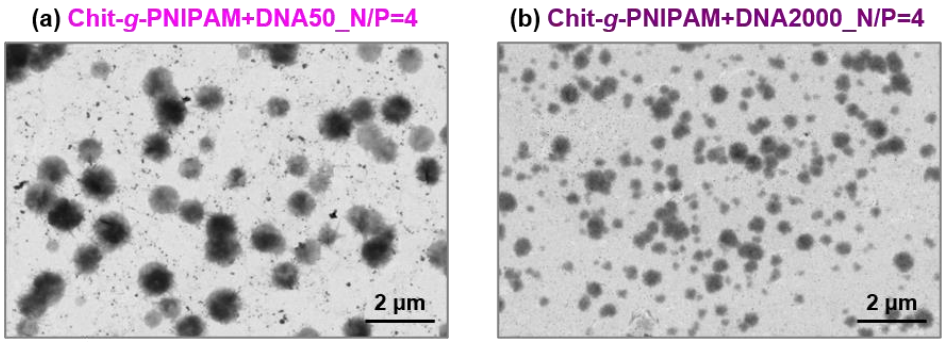

Figure 2. STEM images for representative polyplexes of the two Chit-*g*-PNIPAM+DNA systems. Adapted from [4].

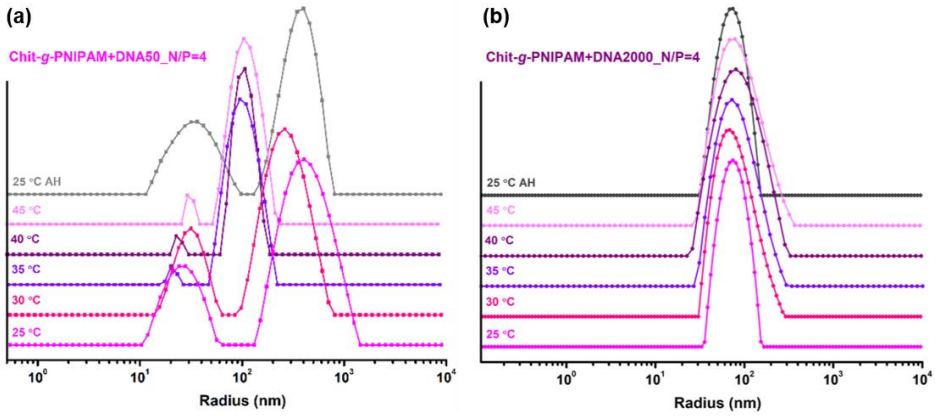

Figure 3. DLS size distributions at different temperatures for representative polyplexes of the two Chit-*g*-PNIPAM+DNA systems. Adapted from [4].

4. Conclusions

The Chit-*g*-PNIPAM copolymer interacts electrostatically with both DNAs, forming stable and thermoresponsive polyplexes, whose physicochemical properties depend on the intrinsic conformation of the graft copolymer, the length of the DNA molecule, and the mixing ratio of the two components.

Acknowledgements

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GREEN SYNTHESIS OF GOLD NANOPARTICLES STABILIZED BY AMYLOPECTIN-*g*-POLY(ACRYLIC ACID) COPOLYMER

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1. Introduction

Gold nanoparticles (AuNPs) are submicrometer-sized gold particles suspended in different fluids (water or organic solvents) that exhibit localized surface plasmon resonance properties and possess exceptional optical and electronic characteristics. AuNPs are inert, non-toxic, and do not react with the body's internal environment making them ideal for medical applications, including drug delivery, photomedicine, biosensing, antimicrobial and anticancer agents. Due to their high surface energy, AuNPs have a tendency to aggregate, making necessary the use of stabilizers or capping agents to ensure their high performance. In the last years, polymers have attracted considerable attention, being used as reducing and stabilizing agents, in order to avoid aggregates during AuNPs synthesis and leading to a better distribution and orientation of the metal particles [1].

Amylopectin (AMP) is a natural polymer with a higher molecular weight, known as the major component found in starch granules (75–85%). AMP, has highly branched structure and is composed of α-D-glucopyranose units linked together by α-(1,4) and α-(1,6)-glycosidic bonds. Among the abundant content in nature, high biocompatibility and biodegradability, AMP containing numerous free hydroxyl groups that may facilitate the synthesis of AuNPs by reduction of Au^{3+} ions to Au^0 and immobilization of Au ions into its network. Moreover, numerous hydroxyl groups facilitate its chemical modification with different synthetic polymers to create hybrid molecules with adapted functionalities [2]. Poly(acrylic acid) (PAA) is a pH responsive anionic synthetic polymer with high water absorption capacity. Due to its outstanding properties, high biocompatibility, nontoxicity, and recyclability, PAA is widely used with polysaccharides for the development of materials suitable for biomedical applications [3]. In this context, the use of hybrid macromolecular materials based on polysaccharides and stimuli-responsive polymers, as stabilizing and coating agents for AuNPs, is identified as a promising approach for the development of smart/responsive hybrid nanomaterials that integrate their functionalities and properties.

2. Experimental

The overall objective of this study was the synthesis and characterization of a new hybrid copolymer based on AMP and PAA, followed by the synthesis of gold nanoparticles mediated by AMP-*g*-PAA.

PAA was synthesized in our laboratory via reversible addition-fragmentation chain transfer (RAFT) polymerization of acrylic acid (AA) following a previously published methodology [4, 5], where AIBN was used as the polymerization initiator and 4-cyano-4-[(dodecylsulfanylthiocarbonyl) sulfanyl] pentanoic acid as the chain transfer agent. Then, the hybrid copolymer AMP-*g*-PAA was synthesized by anchoring of PAA homopolymer chains to the backbone of AMP via a covalent coupling reaction, following the "grafting to" technique, and using potassium persulfate as the radical initiator (Figure 1). After the reaction has occurred, the non-grafted homopolymer chains have been removed by dialysis, and the copolymer has been freeze-dried. ATR-FTIR and ^{1}H-NMR spectroscopy were performed to verify and confirm the structure of the obtained grafted copolymers.

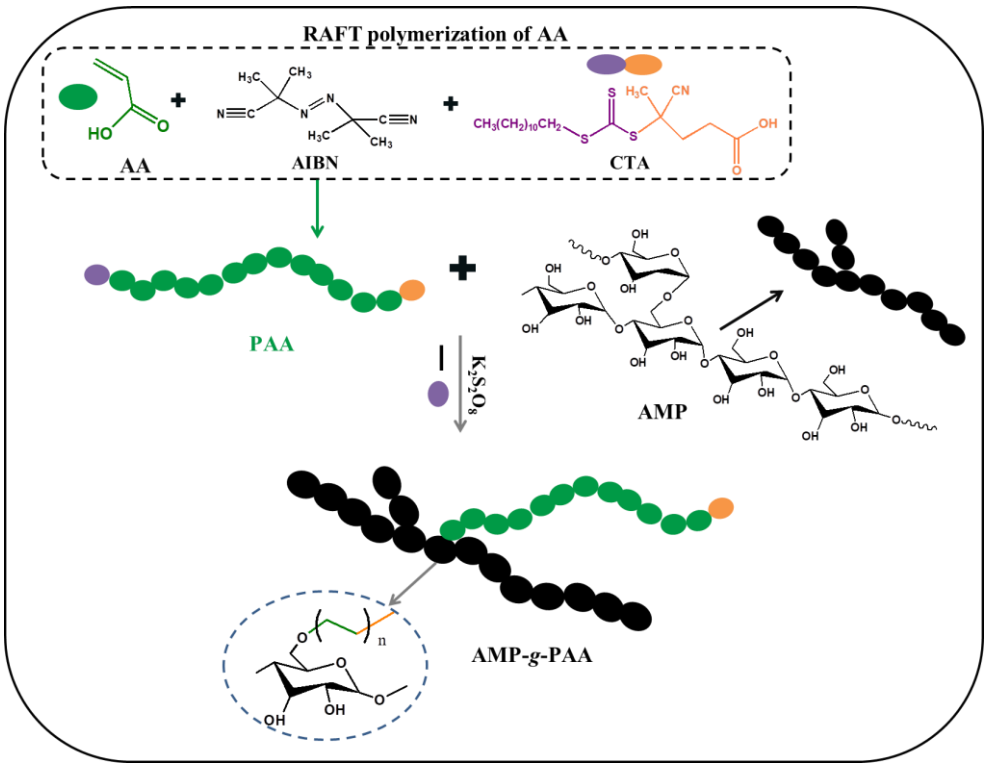

Figure 1. PAA synthesis by RAFT polymerization and "grafting to" synthesis of AMP-*g*-PAA copolymer.

The AMP-*g*-PAA/gold nanoparticles (AuNPs) hybrid composite have been synthesized through a green synthesis method using AMP-*g*-PAA as reducing and stabilizing agent. The AuNPs were formed in aqueous solution using chloroauric acid and AMP-*g*-PAA copolymer in different molar ratios MR = [AMP-*g*-PAA]/[Au], without the use of any additional reducing agents. The samples were heated at different temperatures, in a water bath, and then the solutions were left at room temperature (25 °C). To investigate the kinetics of AMP-g-PAA/AuNPs hybrid nanostructures synthesis in relation to the reaction temperature and molar ratio, UV-Vis measurements, dynamic light scattering (DLS) and scanning transmission electron microscopy (STEM) were performed.

3. Results and discussion

The grafting of PAA chains onto the AMP backbone was confirmed by both ATR-FTIR and ^{1}H-NMR spectroscopies. The spectra obtained for AMP-*g*-PAA displayed the main characteristic signals of AMP and PAA structures. Moreover, from the second derivative of ATR-FTIR spectrum, an increase in the intensity of the alkyl ether bonds (–C–O–C–) stretch was observed in the copolymer spectrum, which suggests the formation of new aliphatic ether bonds by the reaction of –OH groups of AMP with the PAA homopolymer.

The formation of AuNPs in the presence of AMP-g-PAA was confirmed by registering the UV-Vis absorption spectra, following the presence of the peak located at 540 nm (Figure 2). After mixing the samples at specific MR and temperatures, the samples were kept at room temperature, and the UV-Vis measurements were carried out for 10 days, at different time intervals, in order to observe the formation of AuNPs and their stability over time. The UV-Vis absorption spectra revealed that the most efficient formation of AuNPs in aqueous solution occurred at 60 ºC and with a [AMP-g-PAA]/[Au] molar ratio of 2.05.

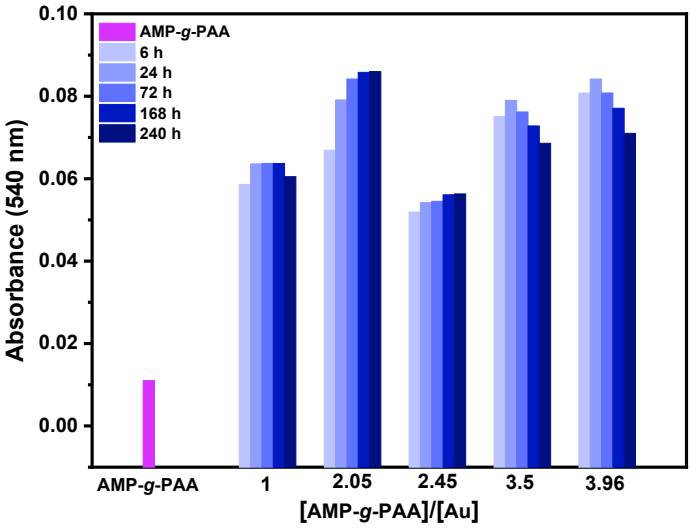

Figure 2. The formation of AuNPs in the presence of AMP-*g*-PAA at 60 ºC and different molar ratios, evidenced by the intensity of the absorbance at 540 nm.

4. Conclusions

In this study, the new hybrid graft copolymer AMP-*g*-PAA was successfully obtained using the "grafting to" method. The formation of the AMP-*g*-PAA copolymer was confirmed by FTIR and ^{1}H-NMR spectroscopic methods. Furthermore, the formation of AuNPs in the presence of a AMP–*g*–PAA, which acts as reducing agent, has been correlated with the [AMP-*g*-PAA]/[Au] ratio, using DLS and UV-Vis absorption techniques.

Acknowledgements

This work was supported by the Romanian National Authority for Research, by project HYBSAC, project number PNRR-III-C9-2022-I8-201, within the National Recovery and Resilience Plan.

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NEW THERMORESPONSIVE COMPOSITES CONTAINING CHITOSAN-*g*-PNIPAM AND *IN SITU* FORMED GOLD NANOPARTICLES

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1. Introduction

Thermoresponsive polymer-coated AuNPs have recently been proven to be attractive materials for the colorimetric sensors since they are particularly sensitive to outside stimuli, including temperature, pH, and salts - all of which are critical indications for biological system monitoring [1,2]. Motivated by this demand, the goal of this research was to create novel AuNPs/smart polymer composite nanostructures with biological applications. This paper offers a novel method for generating AuNPs with diameters smaller than 100 nm utilizing an eco-friendly chitosan-*g*-poly(N-isopropylacrylamide) (Chit-*g*-PNIPAM) thermoresponsive copolymer. This study originality is the one-pot, *in situ* synthesis of AuNPs assisted by an aqueous Chit-*g*-PNIPAM copolymer solution, which requires no extra reducing agent. The Chit-*g*-PNIPAM copolymer was developed using the "grafting to" approach employing a radical-mediated coupling reaction between chitosan and PNIPAM obtained using RAFT polymerization [3]. The kinetics of Chit-*g*-PNIPAM/AuNPs composite structure synthesis were investigated using Scanning transmission electron microscopy (STEM), dynamic light scattering (DLS) and UV-Vis spectroscopy as a function of reaction temperature and amine group/gold ([N]/[Au]) molar ratio and also used to illustrate the thermoresponsive capabilities of the resulting smart nanoparticle colloids.

2. Results and discussion

The optical qualities of AuNPs are significantly influenced by the separation distance, facilitating the monitoring of aggregation using optical properties. Any color transitions from colorlessness to purple are regarded positively, indicating the AuNPs production. After eight days after mixing, the color shift was evaluated as a function of temperature and the [N]/[Au] molar ratio (Figure 1).

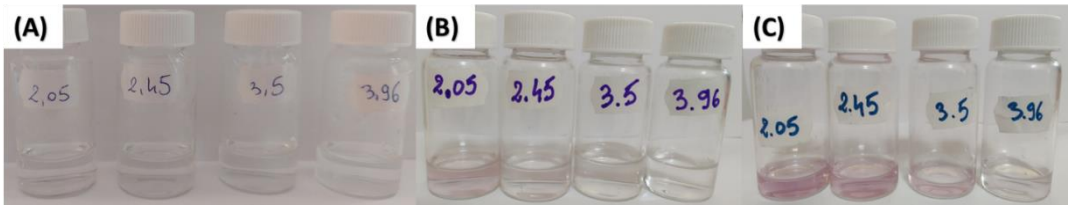

Figure 1. Color visualization of Chit-*g*-PNIPAM/AuNPs after heating at 40 °C (A), 50 °C (B) and 60 °C (C) and 8 days at room temperature.

Figure 1A indicates that the reaction conditions for the samples heated to 40 °C were unfavorable for the *in situ* formation of AuNPs since the color change did not occur, regardless of the [N]/[Au] molar ratio. However, samples exposed to 50 °C and 60 °C show color changes from colorless to various shades of purple after 8 days at room temperature (Figures 1B and 1C). Additionally, the color intensity decreases as the [N]/[Au] molar ratio increases from 2.05 to 3.96 and is dependent on the amount of Au supplied in the reaction media.

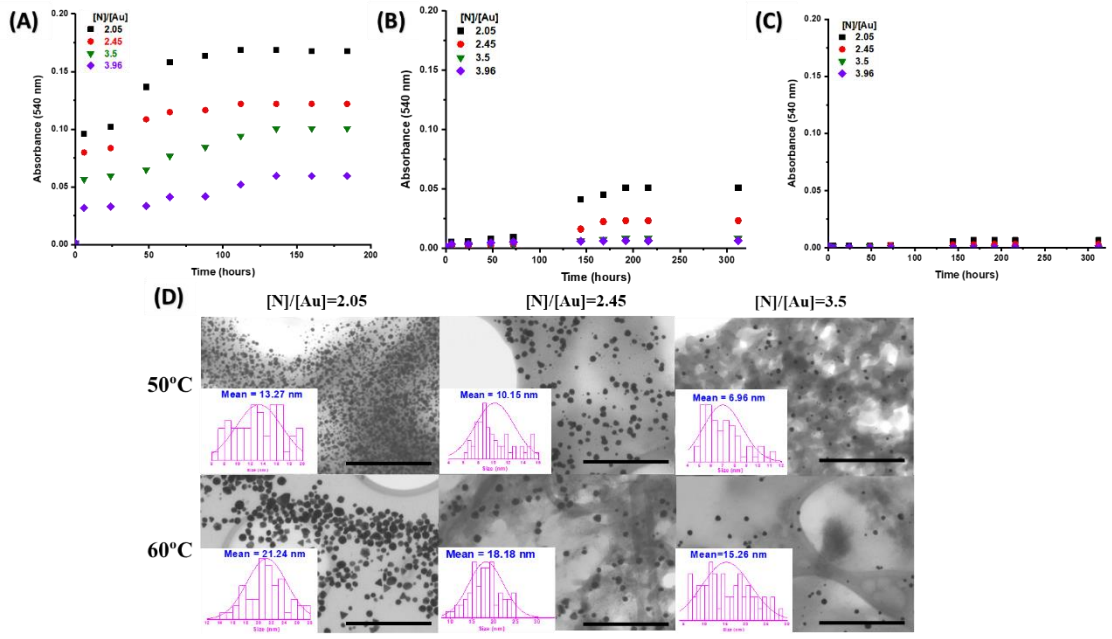

Figure 2. UV-Vis results vs time for [N]/[Au] of 2.05, 2.45, 3.5, and 3.96, heated at 40 (A), 50 (B), and 60 °C (C), and STEM micrographs at 100 nm scale bar (D).

The production of AuNPs may be monitored by localized surface plasmon resonance (SPR), which involves the excitation of free electrons in the conduction band of Au with an absorption peak at approximately 540 nm. The variation of absorbance versus time was used to track the formation of AuNPs (Figure 2). The results indicate that the SPR values at 540 nm varied with temperature and with the [N]/[Au] molar ratio. From STEM images varied mean sizes and mixed geometries (spheres, rhomboids, hexagons, triangles) of AuNPs were found dispersed on the macromolecular matrix, influenced by the preparation conditions. Thus, the reaction temperature and component molar ratio are crucial factors for the synthesis of composite structures in the presence of Chit-*g*-PNIPAM, ruling the size and shape of the in-situ synthesized AuNPs. Additionally, the Chit-*g*-PNIPAM copolymer chains may have dual functions, both as a stabilizer and as a nucleation controller.

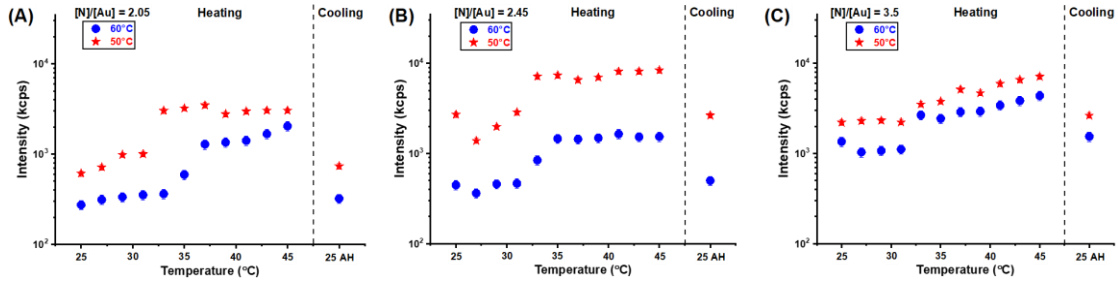

Figure 3. Influence of temperature on the scattered intensity from DLS for Chit-*g*-PNIPAM/AuNPs obtained at 50°C and 60°C and [N]/[Au] of 2.05, 2.45 and 3.5.

Figure 3 illustrates the intensity of the scattered light for Chit-*g*-PNIPAM/AuNPs at different temperatures and [N]/[Au] molar ratio. At temperatures below 35 °C, the low scattered intensity of the Chit-*g*-PNIPAM/AuNPs solution indicates that the majority of the hybrid copolymer chains are free in solution, with just a small proportion of the Chit-*g*-PNIPAM in the aggregates form. The increase in scattered intensity at about 35 °C indicates the formation of aggregated particles with a compact structure. As the temperature increases, the individual chains of the copolymer interact with one another, forming micelle-like aggregates. The intensity in the 35-45 °C temperature range remains constant at 2.05 and 2.45 [N]/[Au] molar ratios (at both reaction temperatures) (Figure 3A and B), indicating unusual stability at such high

temperatures. Above the LCST, the PNIPAM chains from Chit-*g*-PNIPAM/AuNPs tends to avoid water, resulting in the formation of a core-shell structure with a hydrophobic PNIPAM core and a hydrophilic corona. The corona is constructed from Chit-*g*-PNIPAM copolymer which can stabilize the whole structure, proved by low scattering intensity, as compared to the intensity obtained for the Chit-*g*-PNIPAM copolymer [3]. The cationic component of the Chit-*g*-PNIPAM copolymer enables the polyelectrolyte properties, the electrostatic repulsions expanding the temperature range at which the particles are stable. Consequently, there is a competition between the repulsive forces and the associative tendency of the PNIPAM chains. Furthermore, when the [N]/[Au] molar ratio is increased, the intensity starts to rise slowly in the 35-45 °C range, reaching values of about 10^4 kcps at 45 °C (Figure 3C). As a result, the stability of these polymer/Au structures in aqueous conditions is still impacted, and a new aggregation process is occurring. The difference in intensity between the samples obtained at 50 °C and 60 °C are lower as compared to the previously described [N]/[Au] molar ratio. This fact confirms that the stability of Chit-*g*-PNIPAM/AuNPs hybrid system is correlated with the Au concentration in the mixture. Furthermore, the hydrophobic/hydrophilic behavior and aggregation of Chit-*g*-PNIPAM/AuNPs through the LCST appear to be reversible for all the investigated samples.

3. Conclusions

The Chit-*g*-PNIPAM copolymer has been effectively used for the *in-situ* production of AuNPs with varying sizes and thermoresponsive characteristics. The formation of Chit-*g*-PNIPAM/AuNPs composite nanostructures was conducted without an external reducing agent, since the copolymer served as both reducing and protective agent in aqueous solutions. The kinetics and dimension of the AuNPs were associated with the [N]/[Au] molar ratios (2.05; 2.45, 3.5, and 3.96) and reaction temperatures (40, 50, and 60 °C), employing STEM, DLS and UV-Vis methods. Measurements of particle size by DLS were conducted to evaluate the thermoresponsive characteristics of Chit-*g*-PNIPAM/AuNPs nanostructures in aqueous solution, attributed to the presence of PNIPAM chains, with a lower critical solution temperature (LCST) transition occurring at approximately 35°C, near physiological temperature, signifying composite particle aggregation at elevated temperatures. Thus, the synthesized thermoresponsive Chit-*g*-PNIPAM/AuNPs composites may serve as materials in photothermal treatment. Despite the significant promise demonstrated by Chit-*g*-PNIPAM/AuNPs nanostructures, more study is needed to translate them into medicinal applications.

Acknowledgements

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SYNTHESIS AND CHARACTERIZATION OF pH-RESPONSIVE GRAFT COPOLYMER BASED ON POTATO STARCH AND POLY(ACRYLIC ACID)

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1. Introduction

Grafting approach is one of the most attractive methods to develop polymeric materials with improved properties, high performance and sensitivity to stimuli [1]. This study is dedicated to the synthesis of new grafted copolymer based of potato starch (PS) and a poly(acrylic acid) (PAA) obtained by RAFT polymerization, as well as to the structure validation of the grafted copolymer by various methods. PAA is a water-soluble polymer widely used in various fields of applications [2]. Starch is well known for its biocompatibility, biodegradability, low cost and nontoxicity [3].

2. Results and discussion

The synthesis of the new grafted copolymer based on potato starch backbone and poly(acrylic acid) side chains took place in two stages and presented in Figure 1. First, the PAA homopolymer was prepared by RAFT polymerization of acrylic acid (AA) in presence of 4-cyano-4[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CTA) and 2,2'-azobis(2-methyl proprionitrile) (AIBN) (Figure 1A). In the second stage, the copolymer PS-*g*-PAA was synthesized by the "grafting to" approach (Figure 1B) using potassium persulfate (KPS) as initiator.

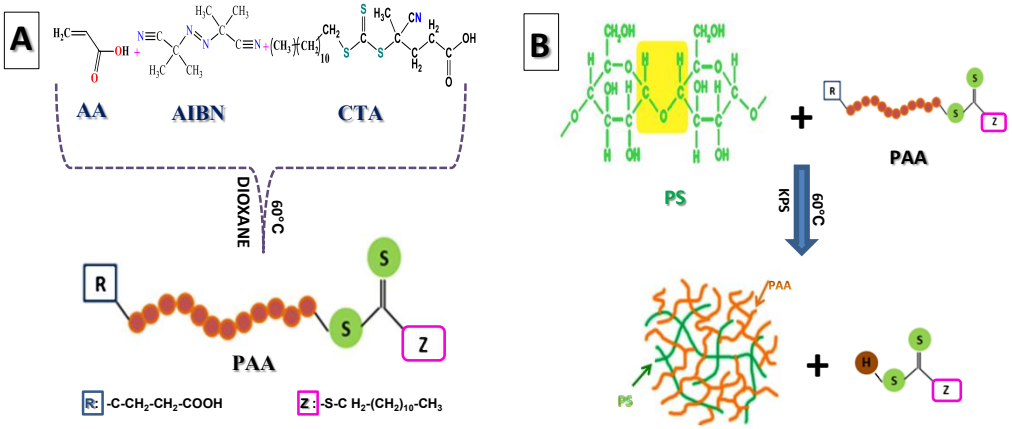

Figure 1. Schematic representation of: (A) RAFT polymerization of AA; (B) grafting reaction of PAA to potato starch.

To certify that PAA side chains were successfully grafted onto PS backbone, the grafted copolymer was characterized by FTIR-ATR and ^{1}H NMR spectroscopies. The FTIR-ATR spectrum of grafted copolymer (Figure 2A) was compared with the spectra of PS and PAA and it can be observed that in the PS-*g*-PAA spectrum the vibration bands corresponding to both starting polymers are present, the strong band at 1718 cm^{-1} being attributed to the –C=O stretching vibration of the carboxyl group in the PAA structure [4]. ^{1}H NMR spectroscopy gives additional information about the structure of the PS-*g*-PAA (Figure 2D) compared with those of PAA (Figure 2C) and PS (Figure 2B). Thus, from the spectrum of the PS-*g*-PAA it can be

observed the signals (1.3-1.8 ppm and 12.8 ppm) characteristic of PAA, and a decrease of the intensity and shift of the signals characteristic for PS, respectively [5]. These features are indicative for successful PAA grafting onto PS backbone.

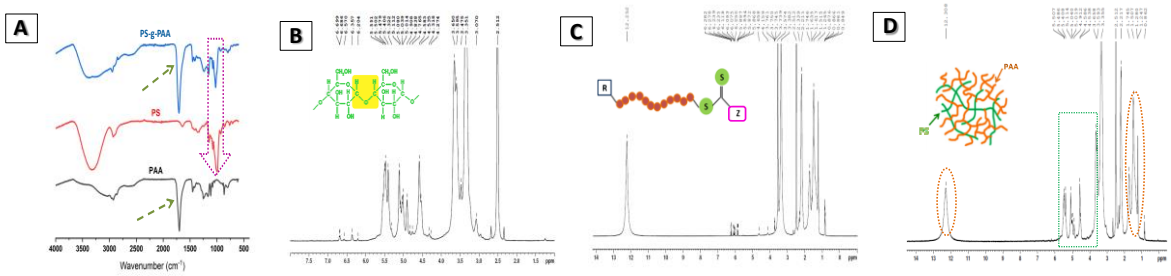

Figure 2. FTIR-ATR (A) and ^{1}H NMR (B-D) spectra of PS, PAA and PS-*g*-PAA.

The behavior of the polymers in aqueous solution can be influenced by various factors, such as polymer structure, molecular weight, pH, concentration or temperature, being very important for various applications such as, drug delivery or wastewater treatment. In this context, the pH-responsive behavior of PS-*g*-PAA in aqueous solution was studied using the viscometry method (Figure 3).

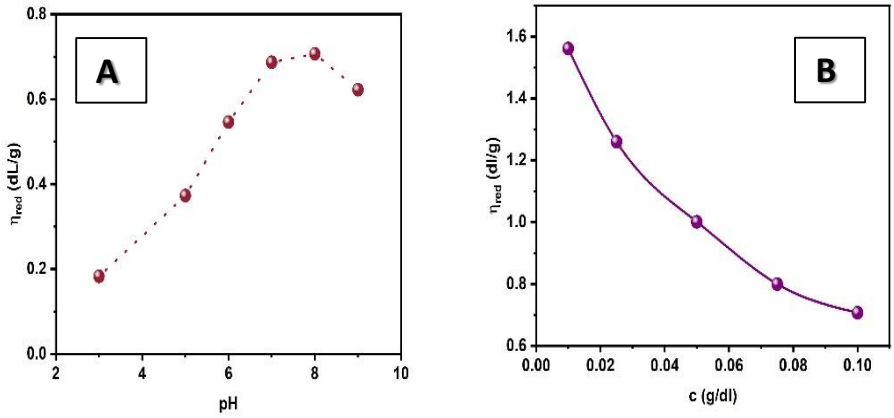

Figure 3. (A) The reduced viscosity *vs* pH values of PS-*g*-PAA aqueous solutions (c=0.1 g/dL) at 25 °C; (B) The reduced viscosity *vs* concentration of PS-*g*-PAA aqueous solutions at 25 °C and pH=8.

The reduced viscosity increases with the increase of pH up to pH = 8 (Figure 3A), after which the value of the reduced viscosity decreases, this behavior being explained by the conformational change of the macromolecular chain. The plots of the reduced viscosity as a function of the PS-*g*-PAA concentration in water (Figure 3B) show a nonlinear shape, which is a typical behavior for polyelectrolyte solutions, i.e., a continuous increase in the reduced viscosity with dilution.

The values of the intrinsic viscosity were estimated by Rao (1) and Fuoss-Strauss (2) equations:

$$\frac{1}{2(\eta_r^{\frac{1}{2}}-1)} = \frac{1}{c[\eta]_R} - \frac{a-1}{2.5} \qquad (1)$$

$$\frac{1}{\eta_{red}} = \frac{1}{[\eta]_F} + c^{1/2} \qquad (2)$$

where: $[\eta]_R$ and $[\eta]_F$ is the intrinsic viscosity determined by the Rao [6] and Fuoss [7] method, respectively, η_r represents the relative viscosity, a is a constant defined as reciprocal of the maximum volume fraction to which particles can pack, η_{red} is the reduced viscosity and c is the concentration of PS-*g*-PAA solution.

The values of the intrinsic viscosity, [η], for the PS-*g*-PAA aqueous solution at 25 °C and pH = 8 are listed in Table 1.

Table 1. Intrinsic viscosity values of PS-*g*-PAA obtained by Rao and Fuoss-Strauss equations.

Sample	$[\eta]_R$	R^2_{Rao}	$[\eta]_F$	R^2_{Fuoss}
PS-g-PAA	1.813±0.006	0.996	4.050±0.0489	0.986

Table 1 shows that there are differences between the [η] values calculated using the Rao and Fuoss equations. These differences may be assigned to the approximations used to calculate the parameters of the equations. Also, the higher values for R^2_{Rao} confirm that the Rao method used for the determination of [η] for the copolymer PS-*g*-PAA solution in water was correctly chosen and shows high reliability.

3. Conclusions

In this study, a two-step process is presented: the first step involves the RAFT polymerization of PAA and the second step is represented by the grafting of PAA onto the PS chain. The chemical structure of PS-*g*-PAA was demonstrated by FTR-ATR and ^{1}H NMR. The viscometric studies provide information about the conformational change of the grafted copolymer macromolecular chains as well as about the pH-sensitive behavior.

Acknowledgements

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NEW POLYSACCHARIDE GRAFTING METHOD PAIRING CHITOSAN WITH PNIPAM BEARING CARBOXYL END GROUP

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1. Introduction

Due to its remarkable biological properties, such as biocompatibility, biodegradability, hemostatic, bacteriostatic, and anticarcinogenic activity, chitosan has become an important component in the preparation of biomaterials and is still being thoroughly studied for use in drug delivery systems [1]. However, its use can be hindered by the limited surface activity/amphiphilicity and heat resistance as well as its poor solubility in basic and neutral solvents, therefore, its physical or chemical modification is very important in order to overcome these disadvantages [2]. Stimuli-responsive polymers are commonly used to impart additional properties to chitosan, such as the ability to react to a range of stimuli, including temperature, pH, mechanical force, electric and magnetic fields etc. Temperature response is by far the most extensively investigated and well-understood. Many polymers exhibit a lower critical solution temperature (LCST), which is the lowest temperature at which phase separation occurs due to a temperature change [3]. Poly(*N*-isopropylacrylamide) (PNIPAM), one of the most widely studied temperature-responsive polymers, exhibits a lower critical solution temperature (LCST) around 32 °C—close to human physiological temperature [4]. In this study, we examine the electrostatic complexation between chitosan (Chit) and the homopolymer PNIPAM that has a chargeable carboxyl end group. We also examined the complexes' temperature responsiveness and evaluated their potential as drug delivery carriers using curcumin (CRC) as the third component of the systems.

2. Experimental

We investigated the formation of polyelectrolyte complexes between chitosan and PNIPAM at different volume ratios (4/1, 4/2, 4/4), while maintaining a constant polysaccharide concentration. Dynamic and electrophoretic light scattering (DLS and ELS) techniques were employed to assess the structural properties of the obtained complexes, focusing on their mass, size and zeta potential. Additionally, changes in mass and size were monitored using DLS to investigate the complexes' thermoresponsive behavior in solution. Lastly, the drug delivery potential of a representative Chit/PNIPAM complex was evaluated by loading curcumin as a model hydrophobic drug at varying concentrations (2.5, 5, and 10% *w/w*) and analyzing the properties of the resulting drug-loaded complexes.

3. Results and discussion

The scattered intensity and the zeta potential were assessed by DLS and ELS measurements and the results are shown in Figure 1. As it can be seen in Figure 1a, the scattered intensity increases significantly with the rising concentration of PNIPAM in the formed complexes. The observed increase provides clear evidence of complexation between the two polymers, given that the scattered intensity is proportional to the mass of the complexes in solution. Figure 1b shows no significant differences in the zeta potential values of the formed complexes, regardless of the PNIPAM concentration. However, a decrease in the effective charge compared to the neat chitosan solution is observed, confirming the interaction between the components.

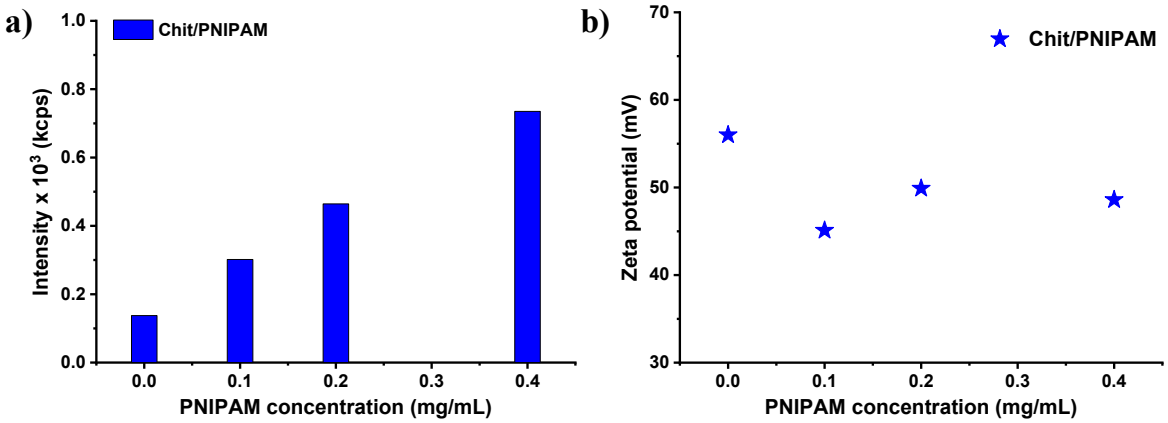

Figure 1. (a) Scattered intensity and (b) zeta potential for the Chit/PNIPAM complexes.

DLS measurements were performed between 25 and 45 °C, with 5 °C increments, to investigate the thermal response of PNIPAM and Chit/PNIPAM 4/1 complex. The effect of temperature on the scattered intensity was examined for the representative Chi/PNIPAM complex at a 4/1 volume ratio and compared with the PNIPAM samples (Figure 2). A distinct increase in turbidity, along a sharp rise in scattered intensity, was observed at 35 °C - above the LCST of PNIPAM. This characteristic reflects the thermoresponsive nature of PNIPAM and is attributed to increased hydrophobic interactions that lead to aggregation between polymer chains. The similar trend observed in both the free PNIPAM and the complex suggests that the thermal phase transition of PNIPAM is preserved upon complexation with chitosan. Importantly, upon cooling to 25 °C, the scattering intensity returns to its original values, indicating that the thermal transition is fully reversible.

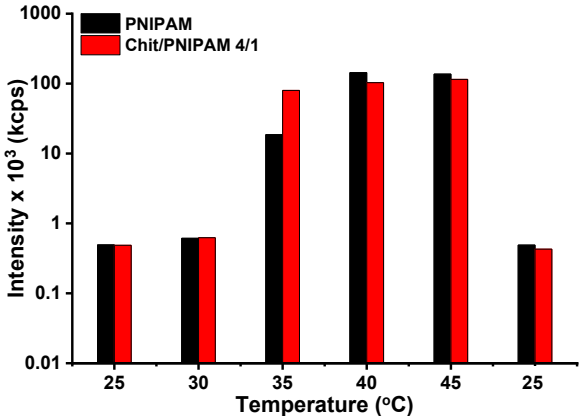

Figure 2. Temperature effect on the scattered intensity for PNIPAM and Chit/PNIPAM 4/1 samples.

The potential application of the obtained Chit/PNIPAM polyelectrolyte complexes as drug delivery systems was investigated by evaluating their encapsulation efficiency of curcumin, a hydrophobic drug. DLS measurements were employed to characterize the physicochemical properties of the CRC-loaded Chit/PNIPAM 4/4 samples. Figure 3 presents the measured scattered intensity values alongside the corresponding average particle sizes. As shown, the DLS results indicate that the CRC-loaded complexes exhibit a significant increase in scattered intensity compared to the unloaded samples (Figure 3a). This increase reflects a higher mass and potentially greater aggregation or complex formation within the nanostructures due to the encapsulation of CRC. Additionally, an increase in the average particle size of the loaded systems is observed (Figure 3b) with increasing curcumin concentration. This increase in size further supports the possibility of aggregation or clustering between the components, likely induced by curcumin incorporation. These variations in scattered intensity and average size indicate an effective drug loading, which changes the hybrid nanostructures' physicochemical characteristics.

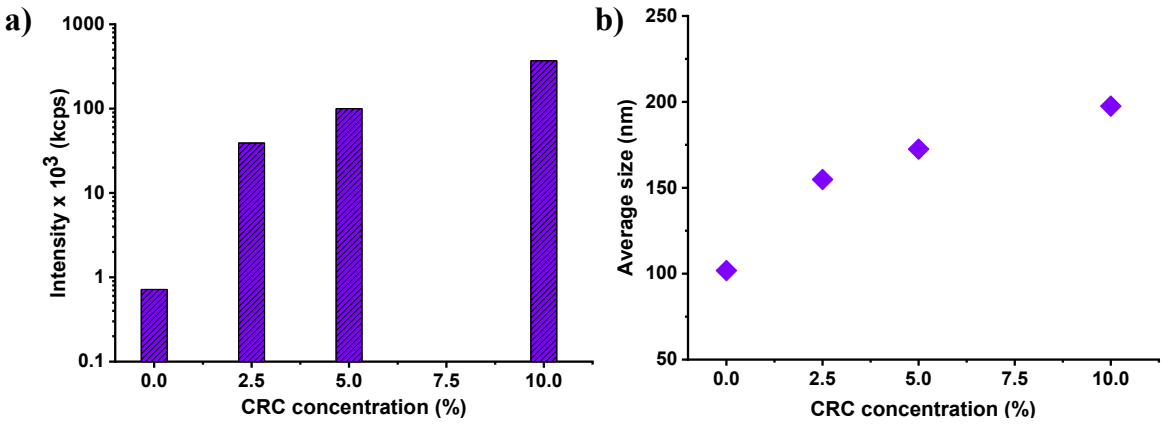

Figure 3. (a) Scattered intensity and (b) average hydrodynamic diameter for the complex Chit/PNIPAM 4/4 loaded with curcumin.

4. Conclusions

Hybrid nanostructures were obtained successfully through complexation method between chitosan and the thermoresponsive homopolymer PNIPAM. The properties in solution for the prepared Chit/PNIPAM systems, regarding the zeta potential, average size and scattering intensity, were assessed by light scattering methods. The temperature response of the Chit/PNIPAM complexes was also evaluated due to PNIPAM's thermoresponsive nature and it was demonstrated that they show similar temperature-dependent behavior above 35 °C. Lastly, the potential use of the obtained nanostructures as drug delivery systems was confirmed through the encapsulation of curcumin, facilitated by the hydrophobic interactions between PNIPAM and the drug.

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POLYSACCHARIDE-BASED (BIO)HYBRID NANOSTRUCTURES

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Polysaccharide based (Bio)Hybrid Nanostructures (HYBSAC) is a project being implemented by the "*Petru Poni" Institute of Macromolecular Chemistry* (ICMPP), Iasi, Romania, project no. 760082/23.05.2023, project code CF201/28.11.2022, funded by the National Recovery and Resilience Plan, Component C9 - Support for the private sector, research, development, and innovation, Investment I3: Development of a program for attracting highly specialized human resources from abroad for research, development and innovation activities.

The goal of the HYBSAC project is to increase the competitiveness of Romanian research, at the national and international level, and build a research core with high-level scientific competence, under the coordination of an international expert, and build a new research field within ICMPP. Top methods will be addressed for synthesizing, characterizing and testing polysaccharides with synthetic polymer components using RAFT polymerization methodologies.

The HYBSAC research project aims to develop new hybrid nanomaterials that will be formed from the combination of natural polysaccharides, synthetic/soluble responsive and biocompatible polymers. The synthesis will be specifically achieved by growing the synthetic polymers in a covalent manner to polysaccharide chains using the RAFT polymerization mechanism. RAFT polymerization is a controlled radical polymerization process that allows for sophisticated tuning of both the structure and functionality of the polymer.

Two synthetic strategies were developed.

- **Grafting-from**, in which polymer grow directly from the polysaccharide backbone.
- **Grafting-to**, in which pre-synthesized polymers will be chemically attached to the polysaccharide.

Either of these approaches was obtain **hybrid synthetic-biological polymers** for advanced functional properties. We will also study the **self-assembly and co-assembly** of these hybrid materials producing well-organized nanostructures through polymer physical chemistry principles, informed by the study of these assemblies in aqueous environments and their structural features and formation mechanisms.

The resultant nanoassemblies was assessed for a variety of high-impact applications such as:

- **Drug delivery, bioimaging and protein transport.**
- **Environmental restoration** as nanocontainers for capturing organic/inorganic pollutants.
- **Surface functionalization** for high-performance material interfaces.

Furthermore, co-assembly with **proteins and antibodies** allow us to create **biofunctional nanoparticles** with biomimetic architecture for diagnostic and treatment purposes. The project also generate **hybrid organic–inorganic and bio–inorganic nanostructures** by co-assembly of functionalized polysaccharides and inorganic nanoparticles, in attempts to create materials that possess **magnetic, optical, catalytic** or **antimicrobial** properties.

The project is coordinated by Dr. Stergios PISPAS, Research Director at the Institute of Theoretical and Physical Chemistry, National Hellenic Research Foundation, Greece (TPCI/NHRF). Dr. Pispas has expertise in polymer synthesis using controlled polymerization techniques, with innovative results applicable in nanomedicine and the delivery of drugs/genes/proteins for therapy, bioimaging, detection, and water treatment. As further evidence of his scientific achievements, Dr. Pispas was included in the *Top 2% Scientists Worldwide in Chemistry* in the field of polymers for the years 2018–2022.

The project team is mainly formed by *Functional Polymers Laboratory* members from ICMPP (https://icmpp.ro/laboratories/l4/description.php), coordinated by Dr. Marcela Mihai, one of Romania's leading research groups with internationally recognized interests on multifunctional (composite) materials mainly through the synthesis and utilization of a variety of synthetic and natural ionic polymers with predetermined functional groups and architectures. In addition, two members of the HYBSAC team originate from TPCI/NHRF, as part of Dr. Pispas's team, to assist with implementing the project. Dr. Pispas' collaborations with Romanian team members date back to 2012 with a number of research visits performed at TPCI/NHRF on subjects aligned with the HYBSAC project.

The project implementation period is 60 months, from July 1, 2023, to June 30, 2026.

The total value of the financing contract is 7,551,991.04 RON, non-reimbursable funds from the European Union – NextGenerationEU, of which the non-reimbursable financing amount is 7,000,000 RON and the VAT related to eligible expenses from PNRR is 551,991.04 RON.

The **general objective** of the HYBSAC project is to increase the capacity and quality of research and development activities at ICMPP by attracting specialists with advanced skills from abroad, opening a new research direction in the field of biomaterials, and creating a research excellence group.

Specific objectives of the HYBSAC project include, but are not limited to:

- Polysaccharides containing rationally designed synthetic polymer constituents obtained by controlled RAFT polymerization. Graft copolymers made with controlled composition and structure. Structural elucidation, self-assembly, and morphology will be evaluated in aqueous media and on substrates.
- Development of biocompatible, synthetic-biological polymer constituents with temperature-triggered and pH-triggered properties that can be co-assembled with biologically relevant materials, namely therapeutic payloads, enzymes, and pre/in-situ created inorganic nanoparticles, for hierarchical control of nanostructures, responsive and environmental conditions, external stimulation, and reaction of the hybrid nanostructures to a specific environmental condition (i.e. those found in living tissue).
- Studying the external controlling/stimulating effects facilitated by the interactions of inorganic components that are responsive, e.g., light and NIR, (ex. gold nanoparticles) embedded in hybrid polysaccharides, or those controllable by an external magnetic field or other forms of radiation (for example, magnetic iron oxide nanoparticles), to regulate their enzymatic activity and analyze/evaluate the combined effects of simultaneous hyperthermic, photothermic, and photodynamic therapy for combined/synergistic therapy and diagnostics.

Specific objectives of the HYBSAC Project (***Aligned with PNRR – Pillar III Strategic Priorities***)

- Enhance the National and International Competitiveness of Romanian Research by establishing a research core with high-level performance indicators (performance center) emphasizing polysaccharide hybrid materials, which was started under the supervision of an internationally renowned researcher and expert, and a new research pathway on hybrid materials based on polysaccharides in high technology. Also, advanced RAFT polymerization techniques have been developed, which will yield polysaccharides with synthetic polymer components to determine the synthesis, characterization, and testing proportions of pure polysaccharide and hybrid polysaccharides and how much of the synthetic polymers are the same.
- Develop the International profile of ICMPP and allow for the new performance Hybrid Materials Research Core to work on EU and national research programs so that it increases the profile of the institution and becomes active in working with other studies.
- Increase the quality and specialization of the human resources by scientific training and collaborative research under different disciplines. The project will be the vehicle where world-class research will be undertaken. It can allow members of the team to acquire competencies as well as improve their practice for those researchers who have more experience in the field of the project.

The activities in HYBSAC will enhance the team knowledge in producing nanomaterials/nanostructures with more desirable properties and functions, greater diversity, and durability. This knowledge will be readily adopted in industry. The group of young researchers (Postdocs and PhD students) engaged in HYBSAC will receive training in a multidisciplinary context within a state-of-the-art research field, concerning a contemporary issue of material sustainability and ecological social development.

Acknowledgements

We also acknowledge the support provided by the Romanian National Authority for Research, with project number PNRR-III-C9-2022-I8-201, within the National Recovery and Resilience Plan.

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